On the Occurrence of Nuclear Dimorphism in a Halteridium parasitic in the Chaffinch, and the probable connection of this parasite with a Trypanosome.

By

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In the course of an investigation on the Hæmatozoa of birds, undertaken as Mackinnon Student, I have recently observed certain forms which are of great importance in reference to Schaudinn's view of the ontogenetic relationship between a Trypanosome and a Halteridium of the "Little Owl."

The material was furnished by a chaffinch, known to be infected with a Trypanosome, which was found to be heavily infected with Halteridium also, towards the end of last June. The preparations of which I shall take account in this note were all made between 1 and 3 a.m.; they comprise smears from the peripheral blood, the heart-blood, and from most of the organs. Unfortunately the lungs were forgotten—an omission which I have deeply regretted. The smears were all well fixed with osmic vapour and stained by some modification of the Romanowsky method. Some of the preparations of the peripheral blood were left for a few minutes before smearing, with or without the addition of a drop of salt-citrate solution. As a result, in these smears free fully-formed microgametes and free rounded-off female elements can be more or less readily found.

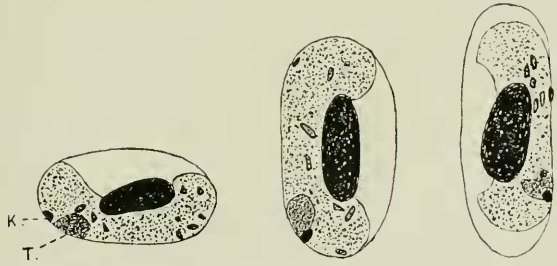
The Halteridia being so numerous in this bird at that time, I confess I expected, from Schaudinn's account, to have little or no difficulty in finding various stages in the transition of the parasites from an active, trypaniform phase, to a resting, intra-cellular, Halteridium-phase and vice-versâ —always supposing, that is, such a connection exists in this case. A general examination of the more likely slides, including smears from the bone-marrow, etc., showed no indications of such behaviour. Trypanosomes of any kind were very scarce as compared with the great number of Halteridia present, and the few individuals noticed were manifestly larger than the largest Halteridia. I was unable to do more than make a somewhat cursory examination at the time, as I was very much occupied with the research in another direction; and owing to the pressure of other work subsequently, it was not until autumn that I was in a position to begin the laborious and time-consuming process of systematically searching these slides. I am pleased to say this study has now yielded me some most interesting results, and further search would yield, I believe, more yet. As, however, I shall probably have to leave the work at this point for some time, I think it worth while to publish this note. I propose to state shortly those observations made up to the present which bear upon the above question, and to consider briefly the meaning which, it seems to me, is to be attached to them.

Fully-grown Halteridia of three types occur, male and female forms with the usual well-known distinguishing features, and a third type corresponding to the "indifferent," or non-sexual form of Schaudinn; the last-named type is distinguished from a female form by its much lighter staining cytoplasm, and from a male form by its compact, denser nucleus. This indifferent type is by far the least common of the three in these slides. Halteridia of all sizes, however, are to be found in the red blood-corpuscles, from very minute, oval or pear-shaped forms, 2 μ or less in diameter, up to the large adult individuals. Many different phases can

often be seen in a few fields of the oil-immersion lens, particularly in liver-smears. A very large number of Halteridia have passed under my eye, but I have never seen the least sign of endogenous multiplication (schizogony) in any of the adult individuals in the red blood-corpuscles, i. e. never anything approaching the nuclear fragmentation and segmentation of the cytoplasm at the two ends, which was described by Labbé.[1] The minute forms do not arise, I am convinced, by the division of the large ones.

Many of the Halteridia exhibit in regard to their nuclear

TEXT-FIGS. 1–3.



1 2 3

Female individuals of Halteridium. 1. From peripheral blood, left a couple of minutes before smearing. 2. From liver-blood; and 3, from peripheral blood (of living bird), both smeared at once. *K*. Kinetonucleus, or kinetonuclear element. *T*. Trophonucleus, or trophonuclear element. Fig. 1 × 2000; Figs. 2 and 3, × 2500.

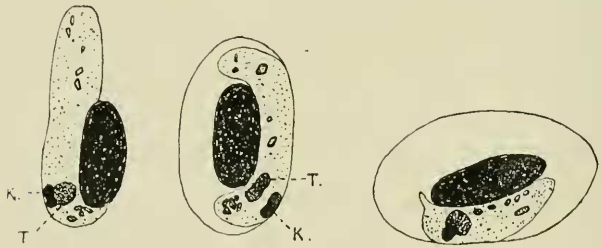
structure a condition which undoubtedly represents nuclear dimorphism. By the term "nuclear dimorphism" is understood a characteristic separation of the nuclear material into two constituents, a larger body staining red with Romanowsky modifications, and a smaller one, which is much denser and stains much darker. These two nuclei, which I have distinguished[2] as tropho- and kineto-nucleus respectively, are of regular occurrence in Trypanosomes and other parasitic

[1] 'Arch. Zool. Exp.,' ser. 3, vol. 2, p. 55, 1894.

[2] "The Hæmoflagellates," 'Quart. Journ. Micr. Sci.,' vol. 50, p. 151, 1906.

Flagellates. I first observed this condition in some of the large female individuals, in which it appears as seen in text-figs. 1–3. Lying close to the ordinary nucleus, generally in contact with it, is a much smaller body, which stains much more deeply than the large one, at times appearing almost black (*K.*). This little body is generally ovoid or round, but in some cases tends to have a rod-like shape. There is no possibility of confusing this nuclear body with a large pigment-grain, or a collection of grains. It lies usually near to the outer surface of the body. In parasites of the indifferent type this nuclear element, which I homologise with a kinetonucleus, is larger

TEXT-FIGS. 4–6.



Indifferent forms of Halteridium. 4 and 5. From heart blood, smeared straightway. 6. From peripheral blood, after addition of a drop of salt-citrate solution, and interval of a couple of minutes before smearing. *K.* Kinetonucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. × 2500.

and may approximate to the size of the other nucleus (trophonucleus). It may be round (text-fig. 6) or, frequently, it is more or less dumb-bell-shaped (text-fig. 5), as if it were composed of two halves. It is important to note that this nuclear dimorphism can be readily recognised in many of the minute forms, the kinetonucleus being a small, deeply-staining grain lying close to the ordinary nucleus, generally at one side (text-fig. 12). This feature gives several of these small individuals a striking resemblance to the resting-phases described of various Herpetomonadine parasites.

In none of the male forms scrutinised so far have I been able to make out a distinct kinetonuclear body. This may be because it is not differentiated, as a compact organella, from the rest of the diffuse nucleus of this type. (I am inclined to think, however, that it is present in the free, fully-formed male gametes, although it is difficult to feel quite sure; I shall refer again to the structure of these delicate elements.) Moreover, in many of the female individuals, the kinetonuclear element is by no means so prominent or separate as in the examples figured, which have been chosen to show this feature as clearly as I have observed it. Others, again, do not show it at all. It is quite probable that at times the two

TEXT-FIG. 7.

Free form, from heart-blood; smear made at once. × 2500. (Probably the upper nuclear body is the kinetonucleus.)

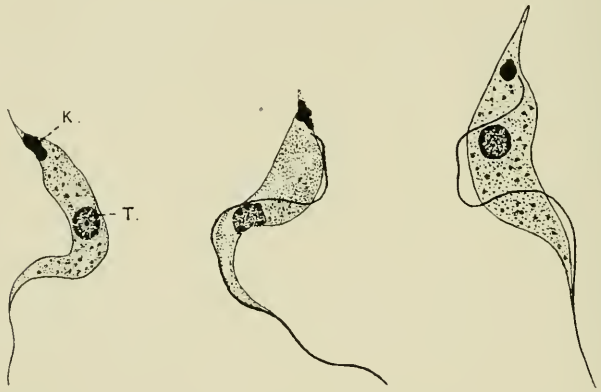
constituents are incorporated in one nucleus, as was said, indeed, by Schaudinn to occur at certain periods.

It is highly significant, I think, that up to the present, when an intra-cellular parasite has been known to exhibit nuclear dimorphism, although lacking in that stage any flagellum or obvious sign of Flagellate affinity, it has been subsequently found to be really a phase in the life-cycle of some Flagellate form; in other words, the knowledge of nuclear dimorphism in a parasite has hitherto heralded, as it were, the discovery of its intimate connection with a Flagellate. The classic instance is the Leishman-Donovan body, whose flagellar phase was first made known by Rogers. Again, Schaudinn himself maintained that certain Piroplasmata showed this character, and others (e.g. Lühe) have since corroborated him. Only recently Miyajima[1] has carried the

[1] 'Philippine Journ. Sci.,' ser. B, vol 2, p. 83, 1907.

matter a step further, and obtained the development of unmistakable Trypanosome phases in cultures from a Piroplasma of cattle in Japan. Hence, the occurrence of this feature in Halteridium even regarded by itself is, to my mind, most suggestive; and it is with very great pleasure that I bring forward what is, I believe, the first definite piece of evidence tending to confirm one, at all events, of Schaudinn's celebrated conclusions.

Text-figs. 8–10.



8 9 10

Trypanosomes from a bone-marrow smear. In 8 the flagellum is very faintly stained, and its course along the side of the body cannot be followed. *K.* Kinetonucleus. *T.* Trophonucleus. × 2500.

Stimulated by this discovery, I have striven to find some phases showing the actual passage from a Halteridium-form to a Trypanosome-form, but in the case of the parasites of the chaffinch such phases appear to be very few and far between. This is, unfortunately, only what might be expected from the great scarcity, comparatively, of the Trypanosomes themselves. I have obtained certain indications, which, so far as they go, point to such a transformation, or fit in with it; and I have observed nothing which in any way invalidates this view.

Before leaving the Halteridium side of the question, there is one phase which I have found, to which I attach con-

siderable importance (text-fig. 7). This is in a smear, well fixed and well stained, which was made from heart-blood and smeared straightway. The parasite is not altered or deformed in any way mechanically; I am confident that it represents a normal phase. The individual in question is free in the blood. It is of the indifferent type, and the two nuclei are in close contact, both dense and deeply staining. The pigment grains are all near one end of the cytoplasm, in

TEXT-FIG. 11.

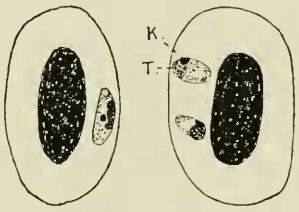
Large Trypanosome, from peripheral blood. × 2500.

a position in which they might easily be got rid of. The most interesting point is the presence of an unmistakable thread or line, which is stained bright red. This starts from a short, transverse, deep-staining band, adjoining the two nuclei, and runs down part of the length of the body near to one side, terminating in a definite granule. The narrow portion of the cytoplasm between it and the margin of the body has a distinctly reddish tinge, the rest of the protoplasm being of the usual blue colour. The only explanation I can give of this thread is that it represents the "central spindle"

described by Schaudinn, which in later development becomes the flagellar border of the membrane, i. e. the proximal part of the flagellum.[1]

Turning to the question of the Trypanosomes, I have now succeeded in finding, in this series of slides, particularly in smears of the bone-marrow, a few very small Trypanosomes, forms which are no larger than the large individuals of Halteridium. When these little Trypanosomes (as in text-figs. 8 and 9) are compared with the large forms in the blood at this time (e. g. fig. 11), the difference in size is seen to be very marked. Between these extremes all intermediate

TEXT-FIG. 12.



Small forms of Halteridium, from a liver-smear. The right-hand figure shows the appearance when two parasites are in one corpuscle, which is of frequent occurrence. *K.* Kineto-nucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. × 2500.

stages can be found. I could figure a series of regular gradations from the one to the other. Now, I have never seen the slightest indications of division; even at this time, when the Trypanosomes are less infrequent than at other times, e. g. early spring or late autumn. Hence, I consider that the larger forms have grown from smaller ones, and not that the small ones have arisen, by successive multiplication, from the large individuals. With regard to the origin of the very small Trypanosomes themselves, I am certainly disposed to think that they arise from adult Halteridia. This seems to me to be the most reasonable conclusion to arrive at, having regard to the data I have ascertained so far.

[1] I have now found this phase in two or three instances.

It is true that the number of Trypanosomes is very small in proportion to that of the Halteridia, but the disproportion is largely reduced if the sexual forms of the intracellular parasites are left out of account; and, as I have mentioned, the great majority of the Halteridia are of the male or female type. It is probable that the Trypanosomes are reinforced in number from the indifferent Halteridia as a rule; and from the female forms only in exceptional circumstances, e. g. perhaps late in the season, if unable to become fertilised. I do not think it likely that the male individuals give rise to ordinary Trypanosomes at all.

In some of my preparations very good examples of micro-

Text-fig. 13.

Male gametes, from peripheral blood, left a couple of minutes before smearing. *b* is the least intensely stained, *c* the most so. *g*. Centrosomic granule. × 2500.

gametes are to be found. These had, in life, freed themselves from the residual body of the gametocyte, were actively motile, and for all I know to the contrary were fully-developed male elements. They have been examined under the best obtainable conditions. This is essential, since the width of these fine organisms is only from ·5 to ·7 μ. Unfortunately, too, the gamete takes up the stain so intensely, that it is often difficult to differentiate sharply between the nuclear and cytoplasmic portions. The chief evidence of trypaniform structure which I hoped to obtain was, of course, the presence of an undulating membrane, as shown by Schaudinn in his schematic figure. I examined, particularly, deeply stained specimens, thinking the flagellar border, standing out from

the body, would be more distinguishable in such. I was not able, however, to satisfy myself of the existence of such an organella. I am uncertain whether there is one or not; it may be that I have not been able to discern it owing to its contiguity to the general cytoplasm.

In certain respects the structure of these gametes does undoubtedly agree with that described by Schaudinn. At one end, which is abruptly rounded, there is an unmistakable granule, which stains more deeply than the neighbouring cytoplasm (text-fig. 13, *g.*). This organella is apparent in most of the individuals observed, and most probably corresponds to the anterior centrosome of Schaudinn. The opposite end is

Text-fig. 14.

Dividing parasite, probably giving rise to small Halteridia; from the bone-marrow. *K.* Kinetonucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. × 2500.

finely tapering, and comparable to the cytoplasmic tail. I have not been able to make out any elaborate nuclear details, but I am inclined to think a kinetonuclear body can be distinguished; at all events, one of the chromatic masses, of which there appear to be three or four, is usually larger and more deeply-staining than the others (cf. fig. 13).

In conclusion, I have a few words to add with regard to the origin of the minute intra-cellular Halteridia. Two or three weeks after this series of preparations was made, I saw for the first time Aragao's account[1] of his work on the Halteridium of the pigeon. Aragao has found that the very young forms, which enter the red blood-corpuscles, originate by the multiple division (schizogony) of large parasites which occur in the endothelial cells of the lung-capillaries. This worker thinks that the form or phase

[1] 'Arch. Protistenk.,' 12, p. 154, 1908.

—whatever it may be—in which the Halteridial parasite enters the blood from the Invertebrate host, in this case a Lynchia, passes first into one of these endothelial cells. In this position it grows and subsequently gives rise to a progeny of little individuals, which, when set free, penetrate the red corpuscles and become the well-known Halteridia.

I have no doubt a similar process occurs in the case of the parasites of the chaffinch. Most unfortunately I omitted to make smears from the lungs. In a smear from the bone-marrow, however, I have come across two instances of a phase which, I believe, corresponds to the segmenting forms described by Aragao. One of these parasites is drawn in text-fig. 14. It is free, having evidently broken loose from the cell, perhaps a leucocyte, in which it was parasitic. It is in process of multiple division, possessing several nuclei. The most interesting point to notice is that some of these nuclei show nuclear dimorphism. Associated with the larger, more obvious nucleus is a small, deeply-staining grain, which probably represents the kinetonuclear element. Two or three of these "double" nuclei closely resemble the double nuclei of the minute Halteridia in the red blood-corpuscles (cf. text-figs. 12 and 14). Unfortunately, the specimen is rather darkly stained, and I cannot be sure of this feature in all the daughter-nuclei.

The connection of Halteridium with a parasite of cells other than red corpuscles (endothelial cells, leucocytes, etc.), made known by Aragao, furnishes another link in the complicated chain of events, which, according to all indications, make up the complete life-cycle of this form. It is instructive to note that Leishmania donovani, which is now admitted by everyone to be the intra-cellular phase of a Flagellate, is a parasite of such cells; and it is not yet certain whether it invades the red corpuscles. Halteridium is probably a stage in the life-history of a Trypanosome, which has advanced a step further and become adapted also to the red corpuscles.

The Lister Institute,
December 10th, 1908.